PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 21/05

A1

(11) International Publication Number: **WO 97/01087**

(43) International Publication Date: 9 January 1997 (09.01.97)

(21) International Application Number: PCT/EP96/02519

(22) International Filing Date: 10 June 1996 (10.06.96)

(30) Priority Data:
1853/95 23 June 1995 (23.06.95) CH

(71) Applicant *(for all designated States except US)*: CIBA-GEIGY AG [CH/CH]; Klybeckstrasse 141, CH-4002 Basle (CH).

(72) Inventors; and

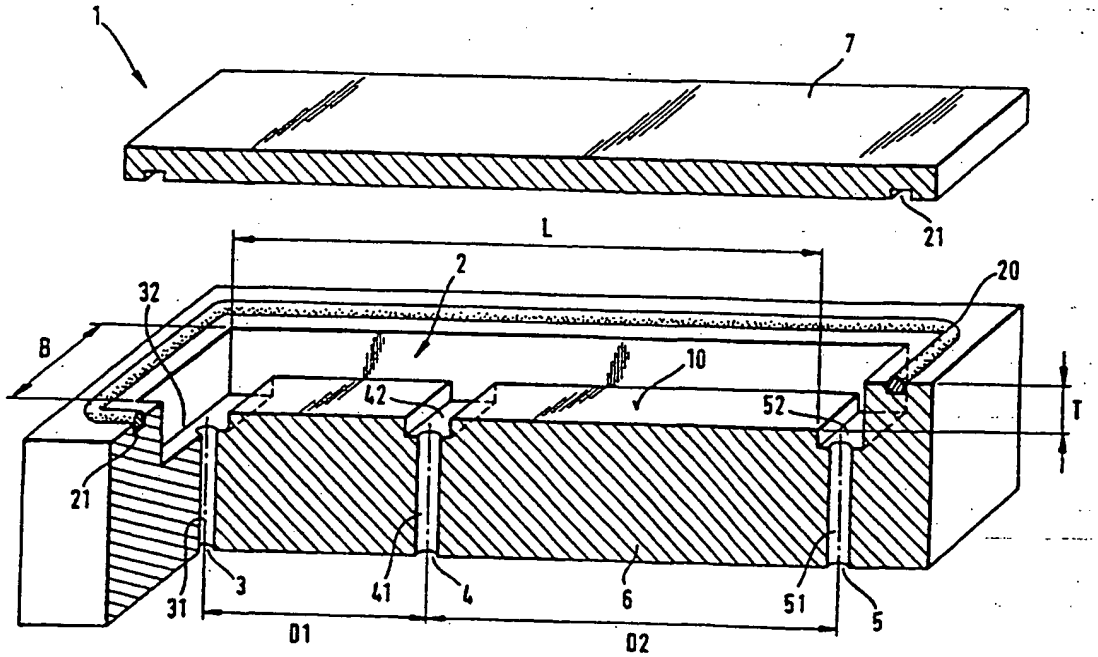
(75) Inventors/Applicants *(for US only)*: OROSZLAN, Peter [HU/CH]; Delsbergerallee 11/3, CH-4053 Basle (CH). ERBACHER, Christoph [DE/DE]; Breddert 64a, D-40723 Hilden (DE). DUVENECK, Gert, Ludwig [DE/DE]; Ezmattenweg 34, D-79189 Bad Krozingen (DE). VERPOORTE, Elisabeth [NL/CH]; Nonnenweg 64 D, CH-4055 Basle (CH).

(74) Common Representative: CIBA-GEIGY AG; Patentabteilung, Klybeckstrasse 141, CH-4002 Basle (CH).

(81) Designated States: AL, AU, BB, BG, BR, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: FLOW CELL



(57) Abstract

A flow cell (1) has in its interior a flow channel (2) and is provided with an inlet opening (3) for the sample and with an outlet opening (4). Also provided are means for producing a sample-free blocked volume located in the flow channel (2). Those means comprise, f r example, a further inlet opening (5) for a reference fluid, that inlet opening (5) being so positioned that the reference fluid flows counter to the sample in the flow channel (2). The flow cell (1) is suitable for a sensor apparatus [illegible] suitable for an optical detection device which [illegible]. The flow cell (1) is also

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America

FLOW CELL

The invention relates to a flow cell according to the preamble of the independent patent claim and to a sensor apparatus and an optical detection device containing such a flow cell. The invention relates also to a method of carrying out analytical measurements on flowable samples and to a method of carrying out optical analytical measurements on flowable samples according to the preambles of the respective independent patent claims.

Flow cells are used nowadays in a large number of analytical measuring systems. Typically, the sample to be investigated passes through an inlet opening into a flow channel contained in the flow cell, flows through the flow channel and leaves the flow cell through an outlet opening. For example, in the flow channel the sample can be subjected to electromagnetic radiation, and the light that is absorbed, transmitted or reflected, or emitted by luminescence excitation, can be analysed by a photoelectric detector, which enables information relating to the composition of the sample and to its physical or chemical properties to be obtained. In another type of measurement, the flow cell is integrated into a sensor apparatus. Typically, one of the boundary surfaces of the flow channel comprises a detection layer in which selectively sensitive recognition elements for analytes that are to be detected are immobilised. When the sample containing the analyte flows through the flow channel, the analyte interacts with the recognition element, thus producing physical or chemical changes in the detection layer or in its immediate vicinity that can be detected by a detector. Those changes may be, for example, of an electrochemical, calorimetric or optical nature. Using electrochemical sensors, for example, it is possible to detect changes in the pH value of the detection layer or to detect electrons released therefrom. Using calorimetric sensors, temperature variations brought about by the analyte/recognition element interaction are detected. In the case of optical sensors, a known method is, for example, to label the analyte to be detected in the sample or the recognition element sensitive thereto in the detection layer using a luminescent dye and to record as a measured variable the luminescence radiation or the change in the luminescence

radiation of the detection layer resulting from the contact between the analyte and the recognition element.

Chemical or biochemical sensor apparatuses containing such sensors are used nowadays in affinity sensing and especially in the context of immunoassays. As (bio)-chemical recognition elements, affinity partners for the analyte to be detected are immobilised in the detection layer; those affinity partners bind the analyte to themselves, for example by means of an antigen/antibody reaction. Efforts are being made in the field of analysis further to miniaturise such sensor apparatuses, that is to say to carry out the measurements on very small volumes of sample, and at the same time further to increase the sensitivity of such sensor apparatuses. In such cases it is desirable for the individual components to be easy to manufacture and, preferably, to be capable of integration in a modular form into relatively complex systems.

The method of evanescent luminescence excitation is known from the prior art, especially in the case of optical sensor apparatuses. In that method, an excitation light is coupled into an optical waveguide surrounded by media of lower refractive index. The excitation light is confined in the optical waveguide by total reflection. In the case of total reflection, a fraction of the excitation light enters the medium of lower refractive index and produces in that medium, in the immediate vicinity of the optical waveguide, the so-called evanescent field. That evanescent field can be used to excite luminescence in the sample. In practice, sensor apparatuses for measuring evanescently excited luminescence are constructed in such a manner that the recognition elements are immobilised on the optical waveguide and the sample is passed over the optical waveguide, for example by means of a flow cell. With a view to the miniaturisation and the modular construction, it is very advantageous in the case of such optical sensor apparatuses for the optical waveguide to be in the form of a planar optical waveguide. The optical waveguide can then function as an integral component of the flow cell, for example as a cover plate for the flow channel. In addition, planar optical waveguides, as opposed to those based on fibre optics, have the advantage that they are significantly easier to manufacture and are thus more suitable for economic mass production. In addition, owing to the planar structure, the manufacture, handling and cleaning of planar optical waveguides are technically considerably less difficult than is the case with the curved surfaces of optical fibres.

An optical sensor apparatus of that type thus typically comprises a flow cell in which one boundary surface of the flow channel is formed by a planar optical waveguide that has a detection layer on the flow-channel side and is applied as a thin layer to a transparent substrate. Frequently, the excitation light is coupled into the optical waveguide by means of a grating contained in the optical waveguide, is confined by the latter and thus excites luminescence in the detection layer *via* the evanescent field. The sample flows through the flow channel, over the detection layer, where the analyte is bound by its affinity partner, over the coupling-in grating, through the outlet opening and out of the flow cell. The coupling of the excitation light into the planar optical waveguide is generally not totally efficient; typically, a portion of the excitation light that irradiates the coupling-in grating is refracted into the flow cell and another portion is coupled into the optical waveguide.

In the case of such optical sensor apparatuses containing planar optical waveguides having a coupling-in grating, serious problems result from the fact that the sample comes into contact with the coupling-in grating. One such problem is that the portion of the excitation light that is not coupled into the optical waveguide but is refracted into the flow cell excites to luminescence those portions of the analyte that are not bound to the detection layer. That can result in an undesired background signal which is virtually indistinguishable from the evanescently excited luminescence of the detection layer. In addition, there is the disadvantageous possibility that the luminescence light not excited in the evanescent field will be coupled into the optical waveguide *via* the coupling-in grating. Another problem is that the contact between the coupling-in grating and the sample has the adverse effect that the refractive index in the region of the coupling-in grating may vary, for example as a result of changes in the composition of the sample. The effective refractive index of the coupling-in grating can also change as a result of particles, for example molecules, contained in the sample being deposited on the grating. That, however, is associated with a change in the angle through which the excitation light is diffracted into the optical waveguide and as a consequence the light intensity coupled into the optical waveguide also varies. Therefore the strength of the evanescent field is also no longer constant, and the measurement is falsified as a result. Those effects thus reduce the accuracy of the measurements, since changes in the strength of the luminescence that are detected

cannot be attributed exclusively to the interaction between the analyte and the immobilised recognition elements in the detection layer. To achieve a marked increase in the sensitivity and the accuracy of such optical sensor apparatuses it is therefore desirable to keep the sample away from the coupling-in grating and to keep the optical conditions over the coupling-in grating constant without adversely affecting the light-guiding properties of the optical waveguide and especially the strength of the evanescent field. Such an adverse effect on the light guiding can arise, for example, from the use of sealing material or structural partitions having a refractive index higher than that of the sample which are in contact with the surface of the optical waveguide downstream of the coupling-in grating in the direction of propagation. A sudden jump in the refractive index can lead to total suppression of further waveguiding downstream of the site of the jump.

A description is given hereinbefore, by way of example, of other, i.e. non-optical, sensor apparatuses comprising a flow cell and a detection layer. In such sensor apparatuses it is likewise often a disadvantage for the sample to be in contact with the whole flow channel. For example, it is then not possible for a portion of the detection layer to be used to generate a reference signal with the aid of which changes in the detected signal caused by interaction between the recognition element and the analyte can be separated from undesired perturbing effects.

It is therefore an object of the invention to make available a flow cell that is suitable for use in sensor apparatuses for analytical measurements on flowable samples and that does not have the mentioned disadvantages. In particular, the flow cell should be so constructed that the sample can be kept away from certain regions of the flow channel without the need for structural partitions that divide the flow channel into a plurality of chambers. The flow cell should be easy to manufacture and suitable for economic mass production, and should also be capable of integration into relatively complex measurement systems of modular construction. The flow cell should also be capable of miniaturisation and should have as little dead volume as possible. It is also an object of the invention to provide an optical detection device into which such a flow cell has been integrated. In particular, an optical detection device having a planar optical waveguide for evanescent luminescence excitation should be improved so that the sample does not come into contact with the coupling-in grating and at the same

time the propagation of light in the optical waveguide, especially its evanescent field, is not adversely affected. A further object of the invention is to propose methods of carrying out analytical and, especially, optically analytical measurements on flowable samples in which the sample is kept away from regions of the flow channel without the need for structural partitions in the flow channel.

Those objects are achieved according to the invention in terms of apparatus and of process technology by the features of the respective independent patent claims. The means for producing the sample-free blocked volume in the flow channel of the flow cell according to the invention comprise, for example, a further inlet opening for feeding in a reference fluid. That inlet opening is so positioned that the reference fluid flows counter to the sample in the flow cell. The sample and the reference fluid are thus introduced at different places in the flow channel, flow towards one another, meet in the region of the outlet opening and leave the flow channel together.

A surprising finding is that, under certain conditions that will be explained hereinafter, the sample and the reference fluid do not mix with one another in the flow channel. As a result, it is possible to keep the sample away from a region of the flow channel simply by means of the counter-flowing reference fluid. That sample-free region occupied by the flowing reference fluid is referred to as the blocked volume. The flow cell according to the invention thus has the advantage that no structural partitions in the flow cell are needed in order to produce the sample-free blocked volume.

Since the flow cell according to the invention has been kept very simple from the point of view of its structure, it can be produced with very little technical effort and is therefore especially suitable for economic mass production. The flow cell can be made, for example, from plastics, for example plexiglass, glass or silicon. The recess for the flow channel can, for example, be milled and the inlet openings and the outlet opening can, for example, be drilled. Also suitable for the production of the flow cell, depending on the material, are electroforming, LIGA processes, lithographic and photolithographic processes, injection-moulding processes and other micro machining processes, such as those used, for example, in microelectronics for structuring semi-conductor materials. Owing to its simple structure, the flow cell according to the invention can also be miniaturised without difficulty. The flow cell can have a very

wide variety of outer forms, for example it may be cuboid or cylindrical, with the result that the flow cell according to the invention is very flexible as regards its range of uses, and in particular can be integrated in a simple manner into relatively complex measurement systems that are preferably of modular construction.

The flow cell according to the invention is suitable, for example, for an optical detection device for analytical measurements on flowable samples. That detection device according to the invention preferably uses evanescent luminescence excitation. In addition to a flow cell according to the invention, it comprises a light source for emitting an excitation light and a photoelectric detector that detects light to be measured coming from the flow cell. Also provided is a source for the reference fluid that can be connected to the inlet opening for the reference fluid. The flow cell preferably comprises a base member one boundary of which has a recess that forms the flow channel, and a cover plate that can be connected to the base member in such a manner that it covers the flow channel. The cover plate comprises a transparent substrate to which a planar optical waveguide has been applied. That optical waveguide has on its surface remote from the substrate a detection layer in which selectively sensitive recognition elements for analytes contained in the sample are immobilised. The cover plate also has a coupling-in grating that couples the excitation light into the planar optical waveguide. The cover plate is connected to the base member of the flow cell in such a manner that the sample in the flow channel is able to come into contact with the detection layer. In that optical detection device according to the invention, the coupling-in grating is so positioned in the cover plate that it is located in the region of the sample-free blocked volume of the flow channel. As a result, only the reference fluid, and not the sample, flows over the coupling-in grating.

That has the advantage that the optical conditions in the vicinity of the coupling-in grating are constant. In particular, a situation is avoided in which particles contained in the sample become deposited on the coupling-in grating and thus alter the effective refractive index of the grating. In addition, variations over time in the refractive index in the region of the coupling-in grating are in practice avoided. As a result, the coupled-in light intensity also varies to an appreciably lesser extent, which leads to a considerable improvement in the constancy of the strength of the evanescent field

and hence to an increase in measurement accuracy. Keeping the sample away from the coupling-in grating also has the advantage that undesired background signals and any coupling thereof into the optical waveguide *via* the coupling-in grating are considerably reduced. Since the sample cannot enter the region of the coupling-in grating, the non-evanescent luminescence excitation of the unbound analyte by the portion of the excitation light refracted into the flow cell is markedly reduced. That also results in greater accuracy and in an increase in the sensitivity of the measuring. Also especially advantageous is the fact that structural partitions in the flow channel that would come into contact with the detection layer and/or the optical waveguide are not needed in order to produce the sample-free blocked volume. Troublesome influences on the propagation of light in the optical waveguide and, especially, on its evanescent field are avoided as a result.

The flow cell according to the invention is, however, also suitable for other, non-optical sensor apparatuses. Such sensor apparatuses comprise, in addition to the flow cell, a detection layer with which the sample in the flow channel can come into contact. The detection layer contains selectively sensitive recognition elements for an analyte. When the analyte binds to the recognition element, physical or chemical properties of the detection layer change and those changes can be detected using a detector. According to the invention, the detection layer is arranged in the flow channel in such a manner that a portion of it is located in the sample-free blocked volume. That has the advantage that a portion of the detection layer, namely the portion in the sample-free blocked volume, can be used to generate a reference signal without the need for structural partitions in the flow channel.

In the method according to the invention for carrying out analytical measurements on flowable samples, the sample and the reference fluid are introduced into the flow channel in such a manner that they flow towards one another. Since, according to the invention, the sample and the reference fluid do not mix, a sample-free blocked volume is produced in the flow channel by means of the reference fluid, without structural partitions being provided in the flow channel. Using that method it is possible, for example, for the sample to be kept away from a portion of the detection layer, even though the whole of the detection layer is in the flow channel. That has the

advantage that the sample-free portion of the detection layer can be used to generate a reference signal.

The method according to the invention for carrying out optical analytical measurements is suitable, for example, especially for exciting luminescence in the evanescent field of a planar optical waveguide and for detecting that luminescence. In that method the excitation light is, for example, coupled into the optical waveguide by way of a coupling-in grating. In that method, the optical waveguide and the coupling-in grating are preferably contained in the flow cell. The sample is brought into contact in the flow channel with the optical waveguide, or with recognition elements immobilised thereon. According to the invention, in that method the excitation light is coupled into the optical waveguide in the region of the sample-free blocked volume. As a result, contact between the sample and the coupling-in grating is avoided. That has the advantages that the optical conditions in the region of the coupling-in grating are constant and that non-evanescent luminescence excitation in the sample is in practice avoided. Since, in addition, there are no structural partitions in the flow channel in contact with the optical waveguide, the adverse effect on the propagation of light in the optical waveguide and especially on the evanescent field is counteracted.

Other advantageous measures and embodiments to which the invention relates are to be found in the dependent patent claims. The invention is explained in detail below, both in terms of process technology and in terms of apparatus, with reference to the drawings. In the diagrammatic drawings, which are not to scale:

Fig. 1 is a perspective sectional view of a first embodiment of the flow cell according to the invention,

Fig. 2 is a plan view of the base member of the first embodiment of the flow cell according to the invention,

Fig. 3 is a plan view of the base member of a development of the first embodiment of the flow cell according to the invention,

Fig. 4 is a section through the base member along the mouth of the discharge channel, corresponding to the line of section IV-IV in Fig. 3,

Fig. 5 is a section, analogous to Fig. 4, but for a variant of the mouth of the discharge channel,

Fig. 6 is a section through a variant of the first embodiment of the flow cell according to the invention with the essential components,

Fig. 7 is a perspective cross-section through a second embodiment of the flow cell according to the invention (without supply and discharge channels),

Fig. 8 is a perspective sectional view of a further variant of the first embodiment (without the cover plate),

Fig. 9 is a plan view of the base member of the variant shown in Fig. 8,

Fig. 10 is a section through a further variant of the first embodiment of the flow cell according to the invention with the essential components,

Fig. 11 is a section through an embodiment of the sensor apparatus according to the invention,

Fig. 12 is a perspective sectional view of an embodiment of the optical detection device according to the invention, and

Fig. 13 is a perspective cross-section through a further embodiment of the flow cell according to the invention (without supply and discharge channels).

A flow cell 1 according to the invention typically has in its interior a flow channel 2 for a flowable sample, an inlet opening 3 for the sample and an outlet opening 4. The expression "flowable sample" is used in the sense that it includes gases and gaseous samples. According to the invention, means for producing a sample-free blocked volume in the flow channel 2 are provided in the flow cell 1. That means that in the

flow channel 2 of the flow cell 1 according to the invention there is at least one region which the sample cannot enter. That region that is inaccessible to the sample is referred to as the blocked volume. The means for producing the sample-free blocked volume in the flow channel 2 especially preferably comprise a further inlet opening 5 (Fig. 1) for feeding in a reference fluid. That inlet opening, referred to hereinafter as the second inlet opening 5, is so positioned that the reference fluid flows counter to the sample in the flow channel 2. The sample and the reference fluid are introduced into the flow channel 2 at different places, flow towards one another in the flow channel, meet in the region of the outlet opening 4 and leave the flow channel 2 together but substantially unmixed.

Fig. 1 is a perspective sectional view of a first embodiment of the flow cell according to the invention which, as a whole, has been given the reference numeral 1. In that first embodiment, the flow cell 1 comprises a cover plate 7 and a base member 6, which is cuboid in form. One boundary surface of the base member 6 has a recess that forms the flow channel 2 for the flowable sample. The base member 6 is additionally provided with the inlet opening 3 for the sample - which is referred to hereinafter as the first inlet opening 3 - and with the second inlet opening 5 for the reference fluid, and with the outlet opening 4. Those three openings 3, 4, 5 are located in that boundary surface of the base member 6 that is opposite the boundary surface having the recess for the flow channel 2. From each of the inlet openings 3, 5 and from the outlet opening 4, a supply channel 31, 51 and a discharge channel 41, respectively, extend through the base member 6 to a mouth 32, 52, 42 into the flow channel 2. The mouths 32, 52, 42 are arranged in such a manner that the mouth 42 of the discharge channel is located between the mouths 32, 52 of the supply channels. The cover plate 7 can be connected to the base member 6 in such a manner that it covers the flow channel 2. Sealing between the base member 6 and the cover plate 7 can be effected, for example, by means of an O-ring 20 which is accommodated in a groove-like recess 21 in the cover plate 7 or in the base member 6.

In the first embodiment of the flow cell 1 according to the invention shown in Fig. 1, the flow channel 2 has a substantially rectangular base 10 and is cuboid in shape. The flow channel 2 has a length L, a width B and a depth T. The mouths 32 and 52 of the supply channels 31 and 51 are arranged on the two shorter sides of the rectangle

of the base 10 of the flow channel 2, and the mouth 42 of the discharge channel 41 is arranged at a distance D1 from the mouth 32 of the first supply channel 31 for the sample and at a distance D2 from the mouth 52 of the second supply channel 51 for the reference fluid. All three mouths 32, 52, 42 are in this case in the form of flat troughs extending parallel to the shorter sides of the rectangle of the base 10 of the flow channel 2 and over the entire width B of the flow channel 2. From the middle of their associated flat troughs, the two supply channels 31 and 51 and the discharge channel 41, each of which is cylindrical in form, lead to the inlet openings 3 and 5 and to the outlet opening 4, respectively. To aid understanding, Fig. 2 also shows a plan view of the flow-channel side of the base member 6 of the first embodiment, which is shown in Fig. 1.

The base member 6 and the cover plate 7 can be made, for example, of plastics, for example plexiglass, glass or silicon. The requisite recesses and bores can be made by milling or drilling or can be produced by means of another method of working known *per se*. Depending on the material, there are suitable, for example, electro-forming, LIGA processes, lithographic and photolithographic processes, injection-moulding processes and other micro machining processes, such as those used, for example, in microelectronics for structuring semi-conductor materials. The sample and the reference fluid are fed by means of a pressure gradient, for example produced using a pump or a syringe, through the first inlet opening 3 and the associated supply channel 31 and through the second inlet opening 5 and the associated supply channel 51, respectively, into the flow channel 2 of the flow cell 1. The sample then flows from the mouth 32 through the flow channel 2 in the direction towards the mouth 42 of the discharge channel 41 and the reference fluid flows from the mouth 52 through the flow channel 2 in the direction towards the mouth 42 of the discharge channel 41, thus forming a counter-flow to the sample. In the region of the mouth 42, the two streams of fluid meet one another and leave the flow channel 2 together through the discharge channel 41. According to the invention, the sample and the reference fluid leave the flow channel 2 unmixed, that is to say at the place where the sample and the reference fluid meet a sharp dividing line is formed between the two streams of fluid. The surprising finding that mixing of the sample and the reference fluid in the flow cell 1 according to the invention can be avoided enables the sample-free blocked volume in the flow channel 2 to be produced by the counter-flowing of

the reference fluid alone and without the necessity for structural partitions in the flow channel. Since the sample and the reference fluid do not mix in the flow channel 2, the sample cannot enter the region of the flow channel 2 occupied by the reference fluid.

Of importance for that flow cell 1 according to the invention is the fact that the geometric dimensions of the flow channel and the average flow rates of the sample and the reference fluid can be matched to one another in such a manner that both the sample and the reference fluid flow through the flow channel 2 in a laminar manner, i.e. without turbulence. It is known that flow remains laminar while the Reynolds number, a dimensionless parameter obtained from the density and the viscosity of the flowing fluid, the average flow rate and a characteristic length determined by the geometric dimensions and the shape of the flow channel, is smaller than approximately two thousand one hundred. At larger values of the Reynolds number the laminar flow is transformed into turbulent flow, which leads to significant mixing of the sample and the reference fluid in the flow channel 2.

In the first embodiment of the flow cell 1 according to the invention having a substantially cuboid flow channel 2 shown in Fig. 1, it has been found that the geometric dimensions and the flow rates can be varied within a wide range without the flow of sample and reference fluid becoming turbulent. The flow rates can without difficulty be as much as 10 cm/s, the width B and the length L of the flow channel 2 can be at least up to 10 cm and the depth T of the flow channel can be at least up to 1 cm. That flow cell is thus very flexible as regards its use and can be adapted without difficulty to the specific requirements of a variety of applications. The flow cell 1 can also be miniaturised without difficulty. In the embodiment shown in Fig. 1, it is possible, for example, to reduce the width B and the depth T of the flow channel 2 to as little as 1 μm. However, those lower limits are not dictated by the counter-flow principle of the flow cell, but result from practical considerations. For the same reasons the flow channel is in practice typically at least 1 mm long. However, smaller dimensions are possible in principle. For practical reasons, values of from 10 μm to 1 mm for the depth T, from 10 μm to 10 mm for the width B and from 3 mm to 40 mm for the length L are preferred.

Especially with a view to their use in miniaturisable measuring devices, it is desirable that optimum use be made for measurement purposes of the volumes of sample located in the flow channel 2, in order to reduce the amount of sample required for the measurement to a minimum. For that reason the dead volume should be as small as possible. Such dead volumes can arise, for example, in corners when sample material or air collects there and becomes "stuck", i.e. does not flow any further. Such effects also extend the time required to fill and/or empty the flow cell and with it the measuring time. In addition, dead volumes can lead to undesired dispersion of the sample. A plan view of a preferred development of the base member 6 intended to minimise the dead volume is shown in Fig. 3. For reasons of greater clarity, a cross-section through that base member 6 corresponding to the line of section IV-IV in Fig. 3 is shown additionally, in Fig. 4. In order to reduce the dead volume, in that development the geometry of the supply channels 31a, 51a and of the discharge channel 41a, and of the respective mouths 32a, 42a, 52a thereof, has been modified. The supply channel for the reference fluid 51a is in the form of a cylindrical bore that extends from the associated inlet opening (which is not shown in Fig. 3 and Fig. 4 but corresponds to the inlet opening 5 in Fig. 1), through the base member 6 and into the base 10 of the flow channel 2. The supply channel 31a for the sample extends in the form of a cylindrical bore from the associated inlet opening (which is not shown in Fig. 3 and Fig. 4 but corresponds to inlet opening 3 in Fig. 1), through the base member 6 and into the plane containing the base 10 of the flow channel 2. From the round opening of the supply channel 31a in that plane, the mouth 32a of the supply channel 31a widens substantially in the form of a funnel in the same plane until it has reached the width B of the flow channel 2 and then merges with the latter. The mouth 42a of the discharge channel 41a into the flow channel 2 (see also Fig. 4) has three branches the ends of which on the flow-channel side are in the form of round openings. Those three openings are arranged on a line parallel to the shorter sides of the rectangle of the base 10 of the flow channel 2. The three mouth branches are so constructed that the two coming from the further outwardly lying openings meet at an oblique angle the mouth branch that continues from the central opening. Inside the base member 6 the ends of the three mouth branches remote from the flow channel 2 unite to form the discharge channel 41a. The latter extends in the form of a cylindrical bore through the base member 6 and ends at the outlet opening 4a.

Fig. 5 shows an especially preferred variant of the form of the discharge channel 41b, or rather of its mouth 42b into the flow channel 2. That mouth is formed by removing the two walls dividing the three branches of the mouth 42a shown in Fig. 4. The mouth 42b of the discharge channel 41b into the flow channel 2 of the variant shown in Fig. 5 is consequently in the form of a slot extending parallel to the shorter sides of the rectangle of the base 10 of the flow channel 2. The length of that slot decreases as the distance from the flow channel 2 increases, until inside the base member 6 the slot merges with the discharge channel 41b. The latter, in the form of a cylindrical bore, then passes through the base member 6 and ends in the outlet opening 4b.

With the flow cell 1 according to the invention, it is therefore possible, by means of the reference fluid in the flow channel 2 flowing counter to the sample, to produce a sample-free blocked volume, without the need for structural partitions in the flow channel 2. It is especially advantageous that the size and position of the blocked volume are controllable. For example, in the case of the cuboid form of the flow channel 2 (see Fig. 1), by altering the distances D1 and D2 between the mouth 42 of the discharge channel 41 and the mouths 32, 52 of the supply channels 31, 51, the size of the blocked volume can be varied and adapted to the requirements of a specific application. The ratio of the distances D1/D2 can be from 0.01 to 100. In addition, it is possible to influence the dividing line or the dividing surface between the two streams of sample and reference fluid. If the sample and the reference fluid are flowing through the flow channel 2 at equal flow rates, a plan view of the flow channel will show a sharp, straight dividing line between the two streams of fluid, which line runs approximately through the middle of the mouth 42 of the discharge channel 41. If, on the other hand, the sample and the reference fluid are flowing through the flow channel 2 at different flow rates, then a curved, but still sharp, dividing line will typically be seen between the two streams of fluid. That effect can be utilised to produce blocked volumes having a curved boundary surface.

Of course the flow cell according to the invention can also be used to produce a plurality of blocked volumes in a single flow channel. That can be effected, for example, by providing further inlet openings and supply channels for the reference fluid and further common outlet openings and discharge channels for the sample and

the reference fluid. For example, in an embodiment similar to the one shown in Fig. 1, the reference fluid can be fed in at both ends of the flow channel and the sample can be fed in between the ends, that is to say, for example, through a channel arranged at the place where, in Fig. 1, the discharge channel 41 is located. A respective common discharge channel for the sample and the reference fluid is again provided between the supply channel for the sample and each of the two supply channels for the reference fluid. With that arrangement, two spatially separate sample-free blocked volumes can then be produced in one flow channel.

A further variant of the first embodiment (Fig. 1) of the flow cell according to the invention that is suitable for the production of a plurality of sample-free blocked volumes in the flow channel 2 is shown in section in Fig. 6, partly symbolically. The line of section corresponds to that in Fig. 1. For the sake of simplicity, only the components essential for understanding are shown in Fig. 6. In that variant, the base member 6 has on its boundary surface remote from the flow channel 2 two inlet openings 301 and 302 for the sample, two inlet openings 501 and 502 for the reference fluid, and four outlet openings 401 to 404. From each of the inlet openings 301 and 302 for the sample a supply channel 310 and 311, respectively, for the sample extends through the base member 6 and opens into the flow channel 2. From each of the inlet openings 501 and 502 for the reference fluid a supply channel 510 and 511, respectively, for the reference fluid extends through the base member 6 and opens into the flow channel 2. In addition, from each of the outlet openings 401 to 404 a discharge channel 410 to 413 leads to the flow channel 2. The flow channel 2 again has a substantially rectangular base 10. At one of the two shorter sides of the rectangle of the base 10 the supply channel 510 for the reference fluid, and at the other the discharge channel 413, opens into the flow channel 2. Between those two channels the two supply channels 310 and 311 for the sample open into the flow channel 2 and between the supply channels 310 and 311 the second supply channel 511 for the reference fluid opens into the flow channel 2. The three discharge channels 410 to 412 are arranged in such a manner that each of them opens into the flow channel 2 between one of the supply channels 310, 311 for the sample and one of the supply channels 510, 511 for the reference fluid.

The reference fluid is fed in through the supply channels 510 and 511 and the sample is fed in through the supply channels 310 and 311. The flow path is shown symbolically by the arrows in Fig. 6. It will be evident from Fig. 6 and the explanations given hereinbefore that during operation of the flow cell two spatially separate sample-free blocked volumes are produced in the flow channel 2. One is located between the mouths of the supply channel 510 and the discharge channel 410 and the other between the mouths of the discharge channel 411 and the discharge channel 412. That variant of the first embodiment of the flow cell according to the invention additionally has the advantage that contact between the sample material fed in through the supply channel 310 and the sample material fed in through the supply channel 311 is avoided. As a result, it is possible to feed two different samples into the flow channel 2 without those two samples coming into contact with one another in the flow channel. A flow cell of that type is suitable, for example, especially for sensor apparatuses and detection devices with which a plurality of samples are to be investigated simultaneously. Of course the flow cell according to the invention can be extended analogously so that there are more than two sample-free blocked volumes in the flow channel. In addition, by means of the suitable arrangement and construction of the supply and discharge channels, the size, the position and the shape of the individual blocked volumes can be controlled.

It will be understood that the cuboid form of the flow channel 2 described hitherto, and its rectangular base 10, are given by way of example. A large number of other geometries for the flow channel 2 are conceivable and can be put into practice without departing from the scope of the invention. For example, it is also possible for the flow channel to have a circular base. The supply channel for the sample can in that case be arranged, for example, in the centre of the circle, the supply channel or channels for the reference fluid at the periphery of the circle and the common discharge channel or channels between the supply channels. The flow channel can, however, have other bases, such as bases that are trapeziform, polygonal, oval, of some other curved outline, or completely irregular.

Of course, the flow cell according to the invention may have more than one flow channel, for example a plurality of parallel flow channels or a plurality of flow channels that diverge from one another in a star-shape. Fig. 7 is a greatly simplified view of a

perspective section through a second embodiment of the flow cell according to the invention. That embodiment has a plurality of flow channels 2a, 2b and 2c. For reasons of clarity, in Fig. 7 the inlet and outlet openings and the supply and discharge channels are not shown. The explanations to be found hereinbefore apply analogously thereto. The line of section in Fig. 7 runs transversely to the flow direction of the sample and of the reference fluid. The base member 6 in that second embodiment has the three parallel flow channels 2a, 2b and 2c. Those channels can be produced, for example, by one of the methods mentioned hereinbefore. In each of those flow channels 2a, 2b and 2c, it is possible in a manner analogous to that described hereinbefore for one flow channel to produce one sample-free blocked volume or a plurality of sample-free blocked volumes. The second embodiment shown in Fig. 7 is also suitable especially for measuring apparatuses with which a plurality of samples can be investigated simultaneously. Another possible application consists in feeding the same sample into all the flow channels 2a, 2b and 2c, but carrying out different measurements in the different flow channels.

A flow cell according to the invention that is functionally equivalent to the second embodiment shown in Fig. 7 can also be produced by constructing the partition walls between the flow channels 2a, 2b and 2c as parts of the cover plate 7. That means that, similarly to Fig. 1, the base member 6 then has only one recess, and the partition walls are produced in the form of projections on the cover plate 7 in such a manner that when the base member 6 and the cover plate 7 have been joined together, they divide the recess in the base member 6 into a plurality of parallel flow channels.

Fig. 8 shows the essential components of a further variant of the first embodiment of the flow cell according to the invention which, from the point of view of its functioning, is very similar to the second embodiment shown in Fig. 7. With that variant it is possible to produce in the flow channel 2 additional blocked volumes that assume the function of the partition walls in the second embodiment (Fig. 7). Means are provided for producing streams of sample flowing adjacent to one another, the streams of sample being separated from one another in each case by a blocked volume.

In the variant shown in Fig. 8, those means comprise a plurality of supply channels 311a, 312a, 313a for the sample and a plurality of supply channels 511a, 512a, 513a

for the reference fluid, which each extend from the associated inlet openings 301a, 302a, 303a and 502a, 503a (the inlet opening of the supply channel designated 511a is not shown in Fig. 8) through the base member 6 to their respective mouths into the flow channel 2. With the exception of the supply channel designated 511a, all the supply channels 311a, 312a, 313a, 512a, 513a are arranged in such a manner that, relative to the direction of flow of the sample in the flow channel 2, they open into the flow channel 2 adjacent to one another, and that a supply channel 512a, 513a for the reference fluid opens into the flow channel 2 between every two supply channels 311a, 312a, 313a for the sample. In a manner analogous to that already explained hereinbefore (see, for example, Fig. 2 and Fig. 3), the supply channels 311a, 312a, 313a, 512a, 513a open into the base 10 of the flow channel 2 at one of the ends of the flow channel 2. The supply channel designated 511a for the reference fluid opens into the base 10 of the flow channel 2 at the opposite end of the flow channel 2. Analogously to the views in Fig. 2 and Fig. 3, the discharge channel 410a in turn opens into the base 10 of the flow channel 2 between the mouth of the supply channel 511a on the one hand and the mouths of the supply channels 311a, 312a, 313a, 512a, 513a on the other. In Fig. 8 and Fig. 9, the mouth of the discharge channel 410a is given the reference numeral 420a.

To aid understanding, Fig. 9 is a diagrammatic plan view of the base member of the variant shown in Fig. 8 in the operational state. Three samples, for example three different samples P1, P2, P3, are introduced *via* the supply channels 311a, 312a, 313a into the flow channel 2 of the flow cell and, as shown by the corresponding arrows, flow in the direction of the mouth 420a of the discharge channel 410a. Reference fluid R1, R2 is introduced into the flow channel *via* the supply channels 512a and 513a and flows parallel to and between the streams of sample, likewise in the direction of the mouth 420a of the discharge channel 410a, as shown by the corresponding arrows. Reference fluid R3 is likewise supplied *via* the supply channel 511a and flows counter to the sample streams P1, P2, P3 and the reference fluid streams R1, R2. The direction of that movement is also shown by a corresponding arrow. In that manner, sample-free blocked volumes can be produced between the individual sample streams P1, P2, P3 in addition to the sample-free blocked volumes between the mouth of the supply channel 511a and the mouth 420a of the discharge channel 410a. Since, as already explained hereinbefore, under laminar flow

conditions the individual streams of fluid do not mix, the streams P1, P2, P3 of sample flowing substantially parallel to one another are effectively separated from one another by the blocked volumes formed by the reference fluid streams R1 and R2, without the need for structural partitions in the flow channel 2. In that manner it is possible, as with the second embodiment shown in Fig. 7, to investigate a plurality of samples simultaneously, or to carry out different measurements on the sample streams P1, P2, P3. Of course, the number of sample streams P1, P2, P3 flowing adjacent to one another and separated from one another by blocked volumes in Fig. 9 is intended purely as an example. It is possible without difficulty also to produce constructions in which fewer or more than three sample streams flow adjacent to one another through the flow channel 2 and are separated from one another in each case by a blocked volume formed by the reference fluid.

Also possible are embodiments in which blocked volumes are produced by the streams of reference fluid only between the individual streams of sample flowing adjacent to one another, and the counter-flowing reference fluid is omitted.

It will be understood that it is, of course, also possible to provide control apparatuses by means of which the flow rates of the sample streams P1, P2, P3 and of the reference fluid streams R1, R2 in the flow channel 2 can be regulated individually.

Of course, it is also possible in the variant shown in Figures 8 and 9 for the base of the flow channel 2 to have a geometry other than the rectangular geometry shown. For example, the base of the flow channel 2 may be trapeziform in shape. In that manner, adjacent sample streams can be produced and at the same time the overall cross-section of all the sample streams can be reduced in size or enlarged along the direction of flow without the individual sample streams mixing with one another.

A further variant of the first embodiment shown in Fig. 1 is shown in a diagrammatic sectional view in Fig. 10. Components that have the same or equivalent functions are given the same reference numerals in Fig. 10 as in Fig. 1. The variant shown in Fig. 10 differs from the embodiment shown in Fig. 1 in that there is provided between the supply channel 31 for the sample and the discharge channel 41 an additional supply channel 51c for the reference fluid which extends from its inlet opening 5c, through

the base member 6 and opens into the flow channel 2 between the mouth of the supply channel 31 for the sample and the mouth of the discharge channel 41. Otherwise the variant shown in Fig. 10 corresponds substantially to the embodiment shown in Fig. 1. In Fig. 10 the operating state of that variant is indicated symbolically. The sample P to be investigated is fed into the flow channel 2 *via* the supply channel 31 and, as shown by the corresponding arrow, flows in the direction of the mouth of the discharge channel 41. The reference fluid R is fed into the flow channel 2 *via* the supply channel 51, flows in the flow channel, likewise as shown by the corresponding arrow, towards the sample P, meets the sample P in the region of the mouth of the discharge channel 41 and leaves the flow channel 2 together with, but substantially unmixed with, the sample P *via* the discharge channel 41. Furthermore, reference fluid is likewise fed into the flow channel 2 *via* the additional supply channel 51c, but flows through the flow channel 2 parallel to the sample P, as shown by a corresponding arrow, without mixing with the sample P, and leaves the flow channel 2 *via* the discharge channel 41. There is thus produced in addition to the sample-free blocked volume between the mouth of the supply channel 51 and the mouth of the discharge channel 41 a further sample-free blocked volume which lies between the mouth of the discharge channel 41 and the mouth of the additional supply channel 51c and between the sample and the boundary surface of the base member 6 facing the flow channel 2. As a result, the sample does not occupy the whole of the depth T (Fig. 1) of the flow channel 2 between the mouth of the additional supply channel 51c and the mouth of the discharge channel 41, as shown in Fig. 10. That variant offers the additional advantage that the thickness of the flowing layer of sample in the flow channel 2 can be altered in a controlled manner, namely by regulating the flow rates. It is also possible with that variant of the flow cell according to the invention to produce extremely thin layers of sample in the flow channel, in a simple manner, by means of the blocked volume located above the sample (with respect to the view in Fig. 10).

It will be understood that the concept on which the variant shown in Fig. 10 is based can also be combined with other embodiments and variants of the flow cell according to the invention. In particular, embodiments of the flow cell according to the invention are possible which combine with one another the principles described with reference to the variants shown in Figs 8 and 9 and in Fig. 10.

The flow cell according to the invention thus has the advantage that in a flow channel having a base of any desired shape a sample-free blocked volume can be produced in any desired position. Since no structural partitions in the flow channel are required for that purpose, the flow cell is simple to manufacture and is thus suitable for economic mass production. The great flexibility of the flow cell in terms of its construction allows it to be used for a large number of applications; it is also especially suitable for integration into relatively complex systems of, for example, modular construction. Since the size, shape and position of the sample-free blocked volume in the flow cell according to the invention can be controlled, the volume of sample in the flow channel that is available for measurements can be optimised, which is of advantage especially for miniaturised measuring apparatuses.

The present invention relates also to the sensor apparatus for analytical measurements on flowable samples into which the flow cell according to the invention is integrated. That sensor apparatus is suitable for a large number of different chemical and physical investigations. Some examples are given hereinbefore. Fig. 11 is a highly diagrammatic representation of an embodiment of the sensor apparatus according to the invention. The flow cell according to the invention has again been given the reference numeral 1. The cover plate 7 of the flow cell 1 has on its surface facing the flow channel 2 a detection layer 8. The detection layer 8 can be brought into contact with a sample P flowing through the flow channel 2 and with a reference fluid R which is likewise flowing through the flow channel.

The detection layer 8 contains recognition elements that are selectively sensitive to an analyte contained in the sample P. Within the context of this invention, the term analyte describes a substance to be detected that may be contained in the flowable sample. Such analytes may be, for example, ions, gases or synthetic or biological compounds. The selectively sensitive recognition elements have the property of binding the analyte to themselves. Such recognition elements may be, for example, enzymes, antibodies, nucleotides, receptor proteins, transport proteins, chelators, indicators or synthetic affinity systems. Such detection layers containing selectively sensitive recognition elements having the property of binding to the analytes are

known *per se* and a large number of them are known, for example, from affinity sensing.

The sensor apparatus according to the invention also comprises a source 100 for the reference fluid R which can be connected to the inlet opening 5 (in Fig. 1). In the embodiment shown in Fig. 11, the connection is made by means of a pipe 101 for the reference fluid. That pipe 101, one end of which opens into the source 100, extends through the inlet opening 5 (see Fig. 1) and the supply channel 51 (see Fig. 1) to the flow channel 2 of the flow cell 1. The reference fluid R passes *via* the pipe 101 from the source 100 to the flow channel 2. The embodiment shown in Fig. 11 also comprises a reservoir 110 for the sample P and a second pipe 111, one end of which opens into the reservoir 110. That second pipe 111 extends from the reservoir 110 through the inlet opening 3 (see Fig. 1) and the supply channel 31 (see Fig. 1) for the sample P to the flow channel 2, with the result that the sample P is able to pass *via* the second pipe 111 out of the reservoir 110 and into the flow channel 2. The discharge channel 41 (see Fig. 1) is provided with an outlet pipe 121 which leads from the flow channel 2 into the space outside the flow cell 1, and through which the sample P and the reference fluid R are able to leave the flow channel 2. The three pipes 101, 111, 121 may, for example, be capillaries. The source 100 for the reference fluid R and the reservoir 110 for the sample P may be, for example, syringes which serve as pumps for the sample P and the reference fluid R. Of course, it is also possible to provide other control apparatuses not shown by means of which the flow rates of the sample and of the reference fluid in the flow channel 2 can be regulated separately. The embodiment shown in Fig. 11 also has two detectors 200 and 210 by means of which particles or waves emanating from the detection layer 8 can be detected or which are capable of measuring chemical or physical variables of the detection layer 8.

During operation, the sample P flows from the reservoir 110 through the second pipe 111 and the flow channel 2, where it comes into contact with the detection layer 8. The reference fluid R flows from the source 100 and the pipe 101 into the flow channel 2 where it flows towards to the sample P. The two fluids P, R meet in the flow channel 2 and leave that channel together *via* the outlet pipe 121. Since the two fluids P, R do not mix in the flow channel 2, there exists in the flow channel 2 the blocked

volume which the sample cannot enter. That is essentially the volume of the flow channel 2 that is occupied by the reference fluid R.

If the sample P contains the analyte to be detected, then when it comes into contact with the detection layer 8 the analyte binds to the recognition element sensitive to it in the detection layer 8. The analyte/recognition element interaction brings about physical or chemical changes in the detection layer 8 which can be detected by means of the detector 210. In the sensor apparatus shown in this case, the detection layer 8 is so positioned that part of it is in contact with the sample-free blocked volume in the flow channel 2. As a result, two regions can be distinguished on the detection layer 8 without the need for structural partitions: one region comes into contact with the sample P but not with the reference fluid R, and the other region comes into contact with the reference fluid R but not with the sample P. Since the reference fluid R ensures that the conditions in the region of the detection layer 8 with which it is in contact are constant, that region can serve as a reference surface which is used by the detector 200 to generate a reference signal. Typically, the two detectors 200 and 210 detect the same physical or chemical measured variable, but the detector 200 detects that originating from the reference surface and the detector 210 detects that originating from the region of the detection layer 8 over which the sample flows. That has the great advantage that changes in the measured variable that result from the interaction between the analyte and the recognition element can be separated from undesired perturbing or background effects. That leads to greater accuracy and sensitivity of the sensor apparatus.

It is also advantageous for a sensor apparatus according to the invention to comprise a flow cell constructed in accordance with the variant shown in Fig. 8 and Fig. 9. That apparatus can be used to investigate a plurality of samples simultaneously, or to carry out a plurality of different measurements simultaneously.

The variant of the flow cell according to the invention shown in Fig .10 also offers further advantages for a sensor apparatus according to the invention. Since very thin layers of sample can be produced therewith in the flow cell 2, the sample can be utilised and investigated markedly more efficiently. When the layers of sample on the detection layer 8 are relatively thick, there is a relatively high degree of probability that

portions of the analyte to be detected will not come into contact with the detection layer 8 at all and as a result will not be detected. If the layer of sample is very thin, however, as can be achieved with the mentioned variant of the flow cell according to the invention, the probability that portions of the analyte will flow past the detection layer 8 "unrecognised" is significantly reduced. The result is a marked reduction in the volume of sample required for the measurement.

Some examples of the sensor apparatus according to the invention follow, in a list that is not exhaustive. In an electrochemical sensor apparatus, the interaction between analyte and recognition element releases electrons which are registered by the detector 210. At the same time, any background signal that may be present is determined by means of the reference surface and the detector 200. In another example, the pH value of the detection layer changes. In a calorimetric sensor apparatus, the temperature in the detection layer 8 changes as result of the contact between the analyte and the recognition element. In an optical sensor apparatus, the detection layer 8 is irradiated with light or another form of radiation. The measured variable is then light or radiation, for example fluorescence light, emanating from the detection layer 8. That light or that radiation is either modified, for example in its intensity, or caused by the binding of analyte and recognition element in the detection layer 8.

The invention relates also to the optical detection device for analytical measurements on flowable samples into which the flow cell according to the invention is integrated, and especially the detection device for measuring luminescence excited in the evanescent field of a planar optical waveguide. Methods and devices for detecting luminescence excited in the evanescent field are known *per se* and therefore require no further explanation. They are used, for example, in affinity sensing and especially in the context of immunoassays. Although the optical detection device according to the invention preferably uses evanescent luminescence excitation, the invention is not limited to the use of that method, but can also be used for carrying out other optical measurements.

Fig. 12 is a diagrammatic representation of an embodiment of the optical detection device according to the invention designed specifically for evanescent luminescence

excitation. In addition to the flow cell according to the invention, which comprises the base member 6 and the cover plate 7, that embodiment has a light source 90 for emitting an excitation light which is shown symbolically by an excitation light beam AL. Also provided is a photoelectric detector 94 which receives light to be measured coming from the flow cell, represented symbolically by beams of light to be measured ML. The detection device additionally comprises a source for the reference fluid which can be connected to the inlet opening 5 in the base member 6. For reasons of clarity, that source is not illustrated in Fig. 12. The source for the reference fluid and the connection thereof to the flow cell can be constructed, for example, analogously to the variant shown in Fig. 11 and the associated explanations relating to the sensor apparatus. Of course, control apparatuses for regulating the flow rates in the flow channel 2 can additionally be provided.

In the embodiment of the optical detection apparatus according to the invention shown in Fig. 12, there is additionally located in the path of the beam of excitation light an optical filter 91 that allows the wavelength(s) of the excitation light to be selected. In the path of the beam between the flow cell and the photoelectric detector 94 there is a lens 92 which collects and focuses the light to be measured coming from the flow cell, and a further optical filter 93 which serves to select the wavelength(s) of the light to be measured that falls on the photoelectric detector 94. Since, usually, the light to be measured, being luminescence light, has a different wavelength from the excitation light that produces the luminescence, the optical filter 93 can be used to reduce the intensity of undesired scattered light, and this has an advantageous effect on the accuracy of the measurement. The light source 90 is preferably a laser or a laser diode.

In that embodiment (Fig. 12) the cover plate 7 of the flow cell according to the invention comprises a transparent substrate 71 to the surface of which on the flow-channel side a planar optical waveguide 72 is applied in the form of a layer. On that optical waveguide, a detection layer 8a is arranged in such a manner that it faces the flow channel 2 and that during operation of the detection device it can come into contact with the sample in the flow channel 2. The detection layer 8a contains immobilised, selectively sensitive recognition elements for analytes contained in the sample, in a manner analogous to that described hereinbefore for the sensor

apparatus according to the invention. The optical waveguide 72 also contains a coupling-in grating 73 for coupling the excitation light coming from the light source 90 into the planar optical waveguide 72. Such planar optical waveguides 72 having a coupling-in grating 73 that are located on the substrate 71 and methods for the production thereof are known *per se* and are described, for example, in EP-A-533 074. There may be used as the material for the substrate 71, for example, glass, quartz or plastics, for example polycarbonate. The material must be transparent at least at the wavelengths of the excitation light and of the light to be measured. The optical waveguide can be produced, for example, from inorganic materials, especially metal oxides, such as TiO_2 or Ta_2O_5.

The flowable sample is fed into the flow channel 2 *via* the inlet opening 3 and the supply channel 31 of the flow cell according to the invention, for example as described hereinbefore for the sensor apparatus. The sample flows through the flow channel 2 and in the process comes into contact with the detection layer 8a. The reference fluid flows out of the source, through the inlet opening 5 and the supply channel 51 and into the flow channel 2 and, in the latter, flows towards the sample. In the region of the mouth of the discharge channel 41, the two fluid streams meet and leave the flow channel 2 together, but substantially unmixed, *via* the discharge channel 41 and the outlet opening 4.

The excitation light coming from the light source is coupled by means of the coupling-in grating 73 into the planar optical waveguide 72, is confined by the latter and thus produces the evanescent field in which the detection layer 8a is located. The evanescent field is able to excite luminescence in the detection layer 8a. The spatially isotropically radiated portion of the luminescence light radiated by the detection layer 8a passes, at least in part, in the form of light to be measured to the photoelectric detector, which converts the light to be measured into a measurement signal which is available for further processing and evaluation. While the sample is in contact with the detection layer 8a, the analyte to be detected, if it is present in the sample, binds to the immobilised recognition element that is sensitive to it. As a result of that binding of the analyte to the recognition element, either the detection layer 8a becomes capable of luminescence for the first time (if, for example, the analyte is luminescence-labelled) or else the luminescence light of the detection layer changes

for example as regards its intensity. That change in the luminescence excited in the evanescent field, or the occurrence of such luminescence, is registered by the photoelectric detector 94.

According to the invention, in the embodiment of the optical detection device shown in Fig. 12, the coupling-in grating 73 for the excitation light is positioned in such a manner that it is located in the region of the sample-free blocked volume of the flow channel 2. As a result, during operation of the detection device only the reference fluid, and not the sample, flows over the coupling-in grating 73. The sample is therefore kept away from the coupling-in grating 73 without the need for structural partitions in the flow channel 2 which would come into contact with the surface of the optical waveguide 72. That measure is advantageous for several reasons. Since only the reference fluid flows over the coupling-in grating 73, the optical conditions in the region of the coupling-in grating 73 are constant. In particular, deposits from the sample on the coupling-in grating 73 and hence changes in the effective refractive index of the coupling-in grating 73 and also variations in the refractive index in the region of the coupling-in grating 73 are avoided, as are variations in the coupling angle through which the excitation light is diffracted during the coupling-in. As a result, the coupled-in light intensity is markedly more stable over time and hence the strength of the evanescent field is considerably more constant. A further consequence is a reduction in undesired background signals and in the coupling thereof into the optical waveguide 72. Since the sample is unable to enter the region of the coupling-in grating 73, there is a marked reduction in the luminescence excitation in the analyte that is excited by that portion of the excitation light that is scattered into the flow channel 2 by the coupling-in grating and is not coupled into the optical waveguide 72 (reduction in the so-called "bulk signal"). As a result, the light to be measured originates practically only from the luminescence excited in the evanescent field. Those effects increase the measurement accuracy and the sensitivity of the detection device. Since no structural partitions are required in the flow channel 2 in order to keep the sample away from the coupling-in grating 73, perturbing influences on the propagation of light in the optical waveguide, for example undesired coupling-out of light, and especially on its evanescent field, are avoided.

In a variant of the embodiment of the optical detection apparatus according to the invention, there is also provided in the optical waveguide 72 a coupling-out grating which is located on the side of the detection layer 8a opposite the coupling-in grating 73 and by means of which light can be coupled out of the optical waveguide. In that case, therefore, the detection layer 8a is located between the coupling-in grating and the coupling-out grating on the optical waveguide 72. Some of the luminescence light emitted by the detection layer 8a is coupled back into the optical waveguide 72. That portion is confined by the optical waveguide 72 and coupled out of the optical waveguide by means of the coupling-out grating, and passes as light to be measured to a further photoelectric detector. It is thus possible to measure both the spatially isotropically radiated portion of the luminescence light emitted by the detection layer 8a and the luminescence light coupled back into the optical waveguide. It is, of course, also possible to measure the coupled-back luminescence only. Alternatively, in that variant the optical detection device may also comprise a flow cell according to the invention and described hereinbefore which can be used to produce more than one blocked volume in the flow channel. That brings with it, for example, the advantage that the coupling-out grating can also be positioned in a sample-free blocked volume.

As already mentioned, the optical detection apparatus according to the invention is not limited to embodiments that make use of luminescence excitation in the evanescent field of an optical waveguide. The detection device can be designed, for example, in such a manner that it contains a flow cell according to the invention without the detection layer 8a, or the light source 90 for transmitting the excitation light that irradiates the flow cell directly, or the photoelectric detector 94 for the light to be measured coming from the flow cell, or the source for the reference fluid, which can be connected to the inlet opening 5. An embodiment of that design is suitable, for example, for detecting as light to be measured the light that is absorbed, transmitted or reflected by the sample in the flow channel 2 or that is emitted by luminescence excitation, in order to derive therefrom information relating to the composition of the sample, or to analyse the properties, especially the optical properties, of the sample. According to the invention, the sample flows only through a portion of the flow channel 2 and only the reference fluid flows through the sample-free blocked volume of the flow channel. That has the advantage that the sample-free blocked volume can

be used to generate a reference signal by means of which perturbing effects, such as variations in the intensity of the excitation light, can be recognised.

Of course, all the variants and embodiments of the flow cell according to the invention described hereinbefore and appropriate combinations thereof can be used for the optical detection device according to the invention and for the sensor apparatus according to the invention. A list of some examples, which is not exhaustive, is given below.

The cuboid shape of the flow channel 2 shown in Fig. 12 is shown here too by way of example. The comments made hereinbefore in connection with the form of the flow channel and the position, size and shape of the sample-free blocked volume or volumes apply mutatis mutandis both to the optical detection device according to the invention and to the sensor apparatus according to the invention.

For example, the optical detection device or the sensor apparatus may contain a plurality of flow channels, especially flow channels that are parallel or that diverge from one another in a star-shape. Suitable for that purpose is a flow cell according to the embodiment shown in Fig. 7 or according to the embodiment shown in Fig. 8 and Fig. 9. The detection layer and the optical waveguide are in that case contained in the cover plate 7, on the flow-channel side. In that arrangement it is possible to provide another detection layer in each of the flow channels 2a, 2b and 2c (Fig. 7) or to provide a single detection layer that comes into contact with all the flow channels. Likewise, the cover plate may comprise a single optical waveguide that extends over all the flow channels, or the cover plate may contain a plurality of spatially separate optical waveguides in the form of strips, each of which comes into optical contact with only one flow channel. That measure makes it possible to investigate one sample using different detection layers, or to investigate different samples simultaneously with the same detection layer.

When a flow cell having a plurality of flow channels, for example parallel flow channels or flow channels that diverge from one another in a star-shape, is used, the above-mentioned influence of structural partitions that come into contact with the surface of the optical waveguide on the light-guiding in the optical waveguide, which

influence is adverse *per se*, can also be used to advantage to reduce the propagation of light transversely to the direction of flow by means of the structural partitions between the individual flow channels. By the selection of materials having a suitable refractive index, that propagation of light can even be almost totally suppressed. The individual flow channels are in practice optically "decoupled" as a result. It is then possible, using a detection device comprising a single optical waveguide and a single detection layer that extend over all of the flow channels, to carry out a plurality of measurements simultaneously without the measurements carried out in different flow channels having any significant effect on each other. The suppression of the light-guiding transversely to the direction of flow of the sample is of course advantageous also when a plurality of spatially separate optical waveguides, arranged, for example, in the form of strips, are provided in the cover plate.

A flow cell according to the variant shown in Fig. 6 can be used, for example, for an optical detection device or a sensor apparatus in which a plurality of spatially separate detection layers are arranged one after another in the flow channel. Of course, the last two examples mentioned can also be combined to form a flow cell containing a plurality of flow channels each of which can contain a plurality of detection layers.

Also especially suitable for the simultaneous investigation of different samples is the variant of the flow cell according to the invention shown in Fig. 8 and Fig. 9 in which the "partition walls" between the streams of sample are provided by blocked volumes produced by the reference fluid. That embodiment has the additional advantage that the blocked volumes functioning as "partition walls" are variable and those "partition walls" can thus be displaced by regulating the flow rates. In that manner, flow patterns can be produced in the flow channel and varied during the investigation. For example, it is possible during the investigation to stop altogether one or two of the streams of sample P1, P2 and P3 indicated in Fig. 9 and simultaneously to increase the flow rates of the adjacent streams. That increase is effected especially advantageously first of all by keeping the overall flow rate of the streams P1, P2, P3, R1 and R2 through the flow channel 2 constant and secondly by keeping the sum of the flow rate of the stream that has been stopped and the flow rates of the two adjacent streams constant. If, for example, the flow rate of the sample stream P2 (Fig. 9) is reduced from 100 % to 0 %, the flow rates of the two adjacent streams of reference fluid R1

and R2 are each increased from 100% to 150%. The fact that it is possible to alter the flow pattern has the advantage that using a single detection layer spatially resolved signals can be obtained. The reference fluid used for producing the blocked volumes between the streams of sample, and hence for producing and altering the flow pattern in the flow channel can be, for example, a light-absorbing fluid. The reference fluid chosen may be, for example, one that is inert towards the detection layer.

Fig. 13 shows yet another embodiment of the flow cell according to the invention that is suitable especially for an optical detection device that utilises luminescence excited in the evanescent field. For reasons of clarity, Fig. 13 does not show the inlet and outlet openings or the supply and discharge channels. That embodiment has only one flow channel 2 which is formed by the recess in the base member 6. The cover plate 7 comprises the transparent substrate 71, and on the side thereof facing the flow channel 2 a plurality of spatially separate parallel planar optical waveguides 721, 722 and 723 arranged as strips. Using the optical detection device according to the invention, which contains a flow cell of the type shown in Fig. 13, different measurements can be carried out simultaneously on one sample. That can represent a considerable saving in time as compared with measurements carried out in succession on the same sample.

Likewise especially suitable for an optical detection device which utilises luminescence excited in the evanescent field is a flow cell according to the invention that corresponds to the variant shown in Fig. 10. With that flow cell, for example, the layer of sample can be made so thin that substantially all of it is in the evanescent field. In addition to the advantages already described, that has the advantage that the non-evanescently excited luminescence is markedly reduced.

What is claimed is:

1. A flow cell having in its interior a flow channel (2) for a sample, and being provided with an inlet opening (3) for the sample and an outlet opening (4; 4a; 4b), wherein means are provided for producing a sample-free blocked volume located in the flow channel (2).

2. A flow cell according to claim 1, wherein the means for producing the sample-free blocked volume comprise a further inlet opening (5) for feeding a reference fluid into the flow channel (2), the mentioned inlet opening (5) being so positioned that the reference fluid flows counter to the sample in the flow channel (2).

3. A flow cell according to any one of the preceding claims, which has a base member (6) one boundary surface of which has a recess that forms the flow channel (2) for the sample, and a cover plate (7) that can be connected to the base member (6) in such a manner that it covers the flow channel (2).

4. A flow cell according to claim 3, wherein the inlet openings (3, 5) and the outlet opening (4; 4a; 4b) are provided in the boundary surface of the base member (6) that is opposite the boundary surface that has the recess, and wherein from each inlet opening (3, 5) and from the outlet opening (4; 4a; 4b) a supply channel (31, 51; 31a, 51a) or a discharge channel (41; 41a; 41b), respectively, extends through the base member (6) to the flow channel (2) and opens into the latter, the mouth (42; 42a; 42b) of the discharge channel (41; 41a; 41b) being arranged between the mouths (32, 52; 32a) of the supply channels (31, 51; 31a, 51a).

5. A flow cell according to claim 4, wherein an additional supply channel (51c) for the reference fluid is provided which extends from an associated inlet opening (5c) through the base member (6) and opens into the flow channel (2) between the mouths of the supply channel (31; 31a) for the sample and the discharge channel (41; 41a; 41b).

6. A flow cell according to any one of the preceding claims, wherein the flow channel (2) has a substantially rectangular base (10) and is preferably cuboid.

7. A flow cell according to claim 6, wherein the mouths (32, 52; 32a) of the supply channels (31, 51; 31a, 51a) are arranged on the two shorter sides of the rectangle of the base (10) of the flow channel (2).

8. A flow cell according to claim 7, wherein the supply channel (31a) for the sample is constructed in such a manner that from the inlet opening (3) for the sample to the plane of the base (10) of the flow channel (2) it is cylindrical in form and its mouth (32a) then widens in that plane to the width (B) of the flow channel (2).

9. A flow cell according to either claim 7 or claim 8, wherein the mouth (42a) of the discharge channel (41a) into the flow channel (2) has three branches, the ends of which on the flow-channel side are in the form of openings arranged on a line parallel to the shorter side of the rectangle of the base (10) of the flow channel (2), and the other ends of which unite again in the base member (6) to form the discharge channel (41a).

10. A flow cell according to either claim 7 or claim 8, wherein the mouth (42b) of the discharge channel (41b) into the flow channel (2) is in the form of a slot parallel to the shorter side of the rectangle of the base (10) of the flow channel (2), the length of the slot decreasing as the distance from the flow channel (2) increases and the slot thus merging with the discharge channel (41b).

11. A flow cell according to any one of claims 6 to 10, wherein the flow channel (2) has a depth (T) of from 1 μm to 10 mm, especially from 10 μm to 1 mm.

12. A flow cell according to any one of claims 6 to 11, wherein the flow channel (2) has a width (B) of from 1 μm to 10 cm, especially from 10 μm to 10 mm.

13. A flow cell according to any one of claims 6 to 12, wherein the flow channel (2) has a length (L) of from 1 mm to 10 cm, especially from 3 mm to 40 mm.

14. A flow cell according to any one of the preceding claims, which contains a plurality of flow channels (2a, 2b, 2c) which are especially parallel.

15. A flow cell according to any one of the preceding claims, wherein means for producing streams of sample flowing adjacent to one another are provided, the streams of sample being separated from one another in each case by a blocked volume.

16. A sensor apparatus for analytical measurements on flowable samples (P), having a detection layer (8) with which the sample (P) can come into contact and which, further, contains selectively sensitive recognition elements for an analyte, which sensor apparatus comprises a flow cell (1) according to any one of claims 2 to 15, and in which sensor apparatus one of the boundary surfaces of the flow channel (2) comprises the detection layer (8), and, further, a source (100) for the reference fluid (R) is provided which can be connected to the inlet opening for the reference fluid (R).

17. An optical detection device for analytical measurements on flowable samples, comprising a flow cell for the sample, a light source (90) for emitting excitation light that irradiates the flow cell, and a photoelectric detector (94) for light to be measured coming from the flow cell, wherein the flow cell is constructed in accordance with any one of claims 2 to 15 and, further, a source for the reference fluid is provided which can be connected to the inlet opening (5) for the reference fluid.

18. A detection device according to claim 17, wherein in the path of the beam between the light source (90) and the flow cell a filter (91) for the selection of the wavelength or the wavelength range of the excitation light is provided.

19. A detection device according to either claim 17 or claim 18, wherein in the path of the beam between the flow cell and the photoelectric detector (94) a filter (93) for the selection of the wavelength or the wavelength range of the light to be measured is provided.

20. A detection device according to any one of claims 17 to 19, wherein the light source (90) is a laser or a laser diode.

21. A detection device according to any one of claims 17 to 20, wherein the cover plate (7) has on its side facing the flow channel (2) a detection layer (8a) which contains immobilised, selectively sensitive recognition elements for analytes contained in the sample and with which the sample in the flow channel (2) can come into contact during operation of the detection device.

22. A detection device according to any one of claims 17 to 21, wherein the cover plate (7) comprises a transparent substrate (71) to the boundary surface of which facing the flow channel (2) a planar optical waveguide (72) is applied, and the cover plate (7) also comprises a coupling-in grating (73) which couples the excitation light coming from the light source (90) into the optical waveguide (72).

23. A detection device according to claim 22, wherein the cover plate (7) comprises a coupling-out grating that couples light out of the optical waveguide (72).

24. A detection device according to either claim 22 or claim 23, wherein the cover plate (7) contains a plurality of planar optical waveguides (721,722,723) which are especially parallel.

25. A detection device according to either claim 22 or claim 23, wherein the cover plate (7) comprises a single optical waveguide that covers all of the flow channels.

26. A method of carrying out analytical measurements on flowable samples in which the sample is introduced through an inlet opening into a flow channel contained in a flow cell, flows through the flow channel and is discharged through an outlet opening, in which method physical or chemical changes in a detection layer of the flow channel are brought about by an analyte contained in the sample and in which method those changes are detected by a detector, which method comprises introducing a reference fluid through a further inlet opening into the flow cell where it flows in such a manner, especially counter to the sample, that the sample is kept away from a blocked volume of the flow channel.

27. A method of carrying out optical analytical measurements on flowable samples in which the sample is introduced through an inlet opening into a flow channel contained in a flow cell, flows through the flow channel and is discharged through an outlet opening, in which method the flow cell is irradiated with excitation light and in which method, further, light to be measured coming from the flow cell is detected photo-electrically, which method comprises introducing a reference fluid through a further inlet opening into the flow cell where it flows in such a manner, especially counter to the sample, that the sample is kept away from a blocked volume of the flow channel.

28. A method according to either claim 26 or claim 27, wherein the sample and the reference fluid are discharged together, but substantially unmixed, through the outlet opening of the flow cell.

29. A method according to either claim 27 or claim 28, wherein the excitation light is coupled by means of a coupling-in grating in the region of the blocked volume of the flow channel into a planar optical waveguide and is confined by the latter, and wherein the sample in the flow channel is brought into contact with that optical waveguide, luminescent substances in the sample or luminescent recognition elements immobilised on the optical waveguide being excited to luminescence in the evanescent field of the optical waveguide, and that luminescence being detected as light to be measured.

30. A method according to claim 29, wherein both the spatially isotropically radiated portion of the luminescence light and the luminescence light coupled back into the optical waveguide are detected as light to be measured.

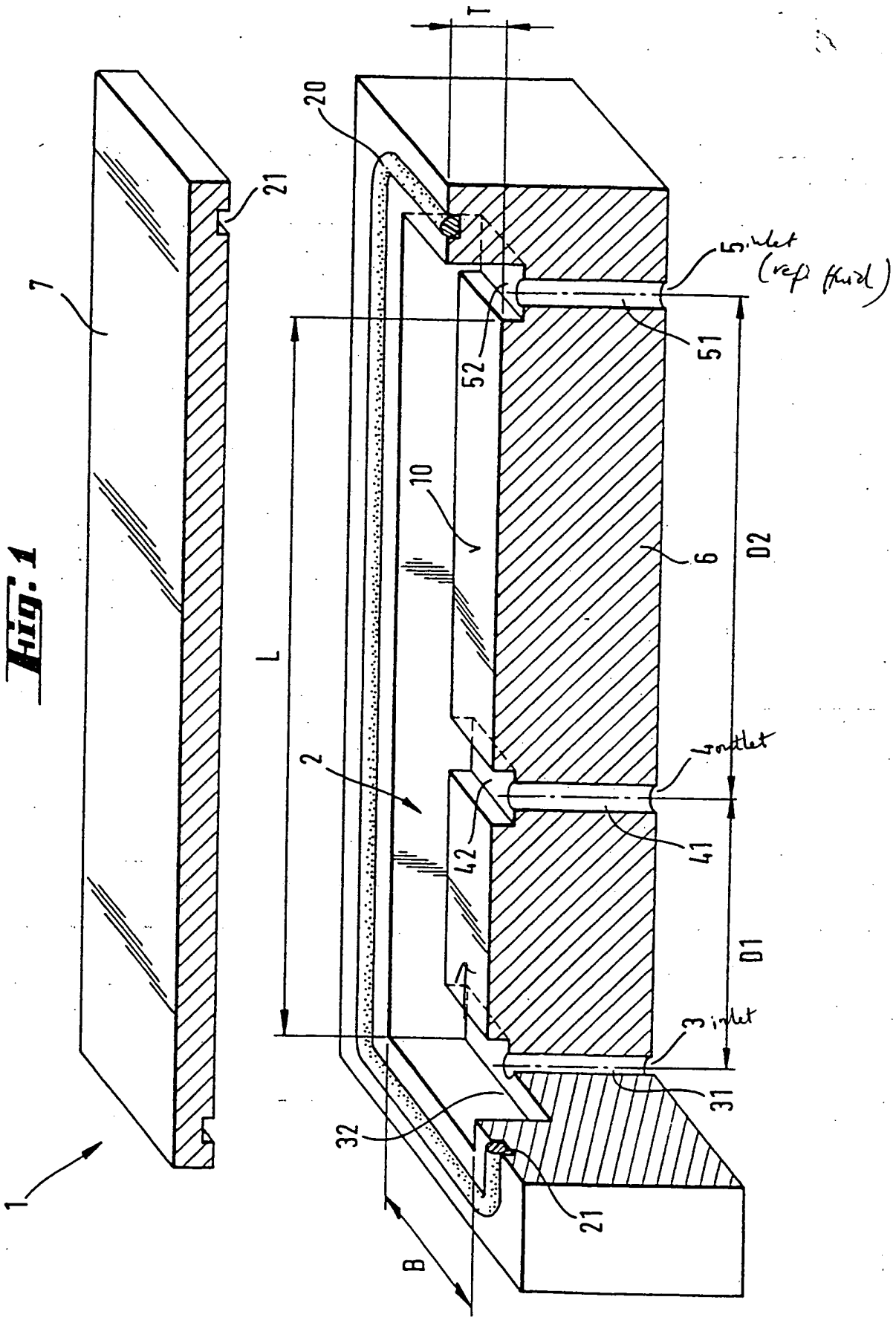

T
20
21
7
5 inlet (cap fluid)
51
52
10
6
D2
L
2
4 outlet
42
41
D1
3 inlet
31
32
21
B
1

Fig. 1

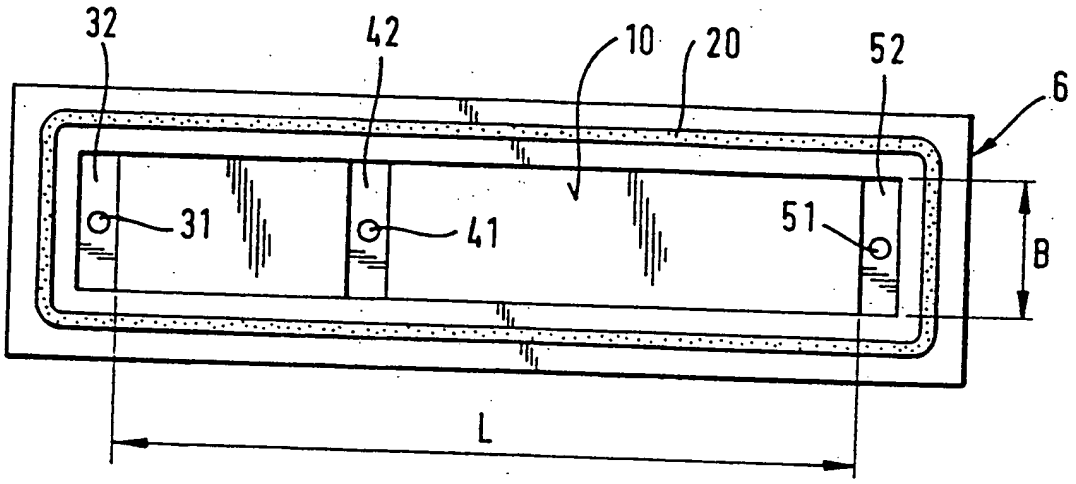


Fig. 2

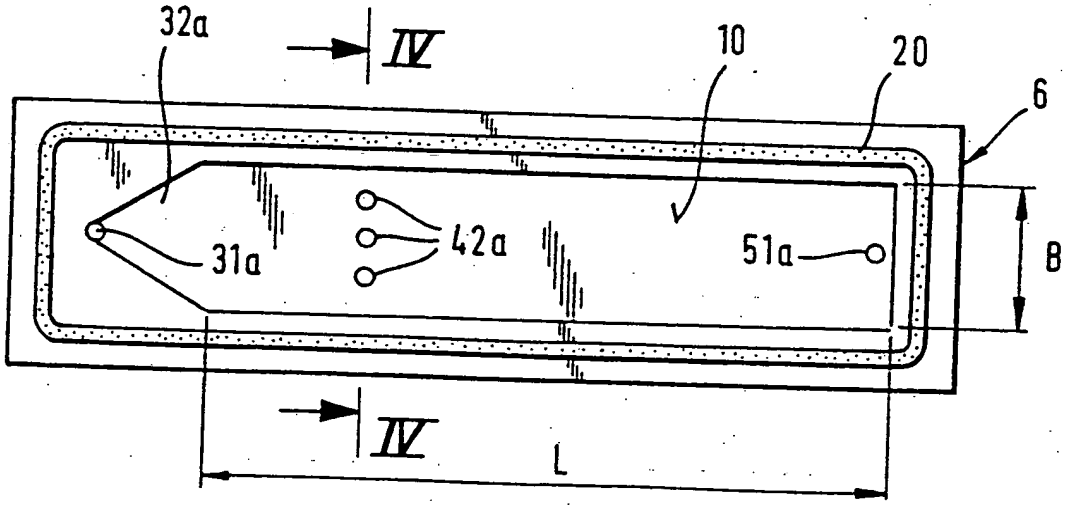


Fig. 3

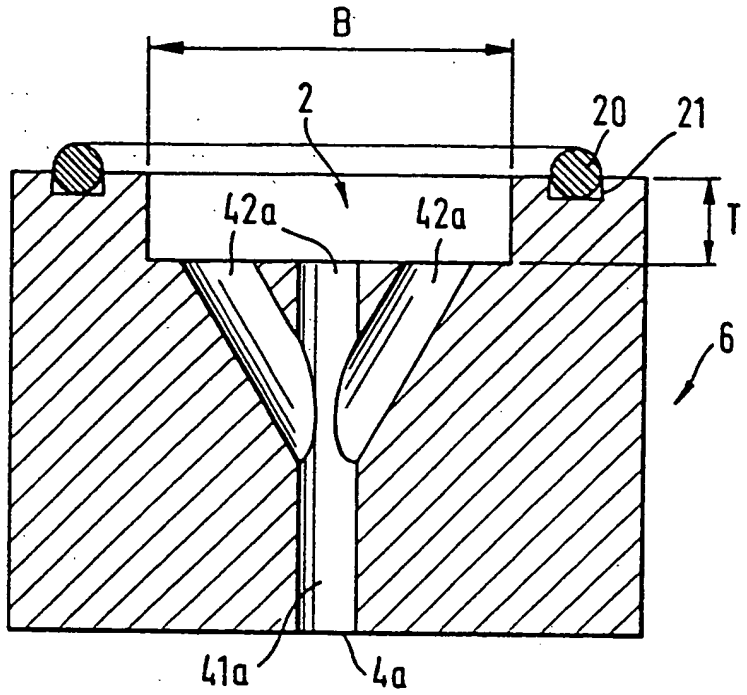


Fig. 4

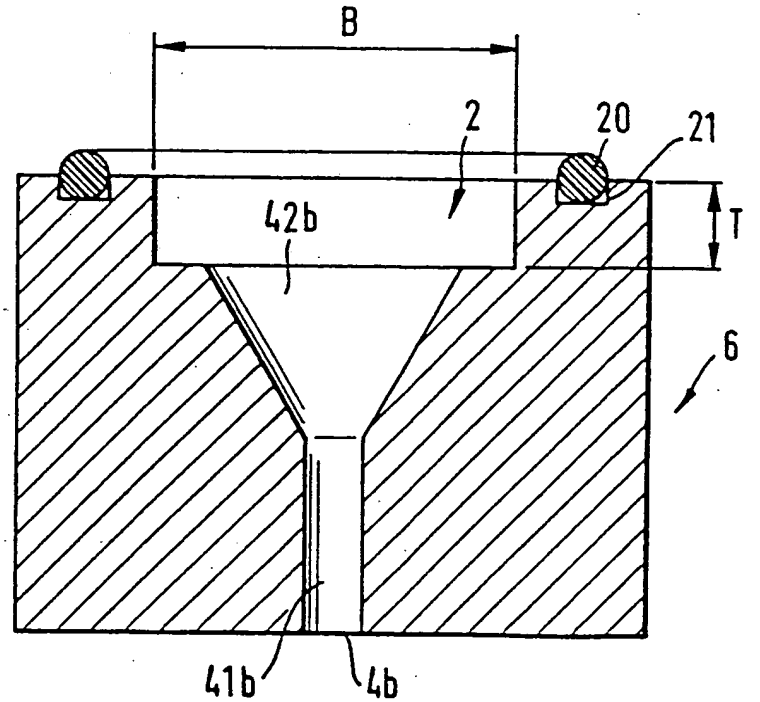


Fig. 5

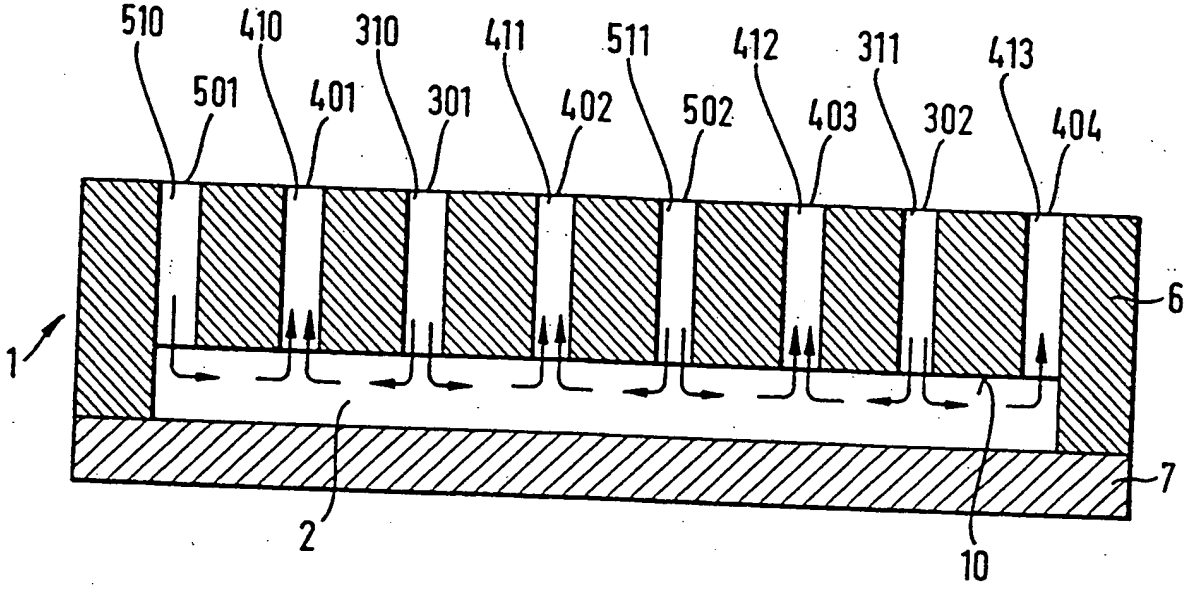

510
410
310
411
511
412
311
413
501
401
301
402
502
403
302
404
1
6
7
2
10

Fig. 6

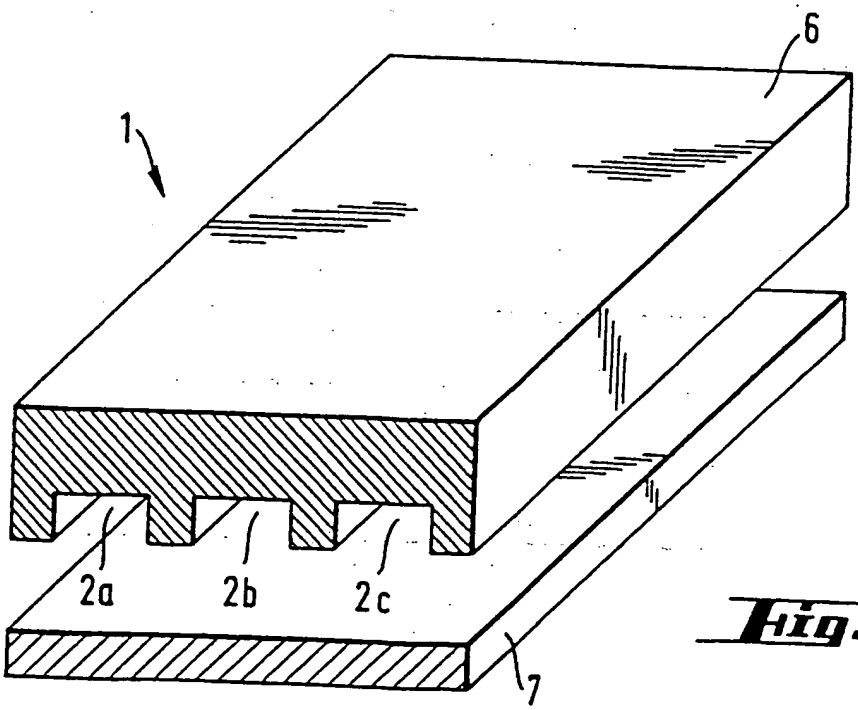

6
1
2a
2b
2c
7

Fig. 7

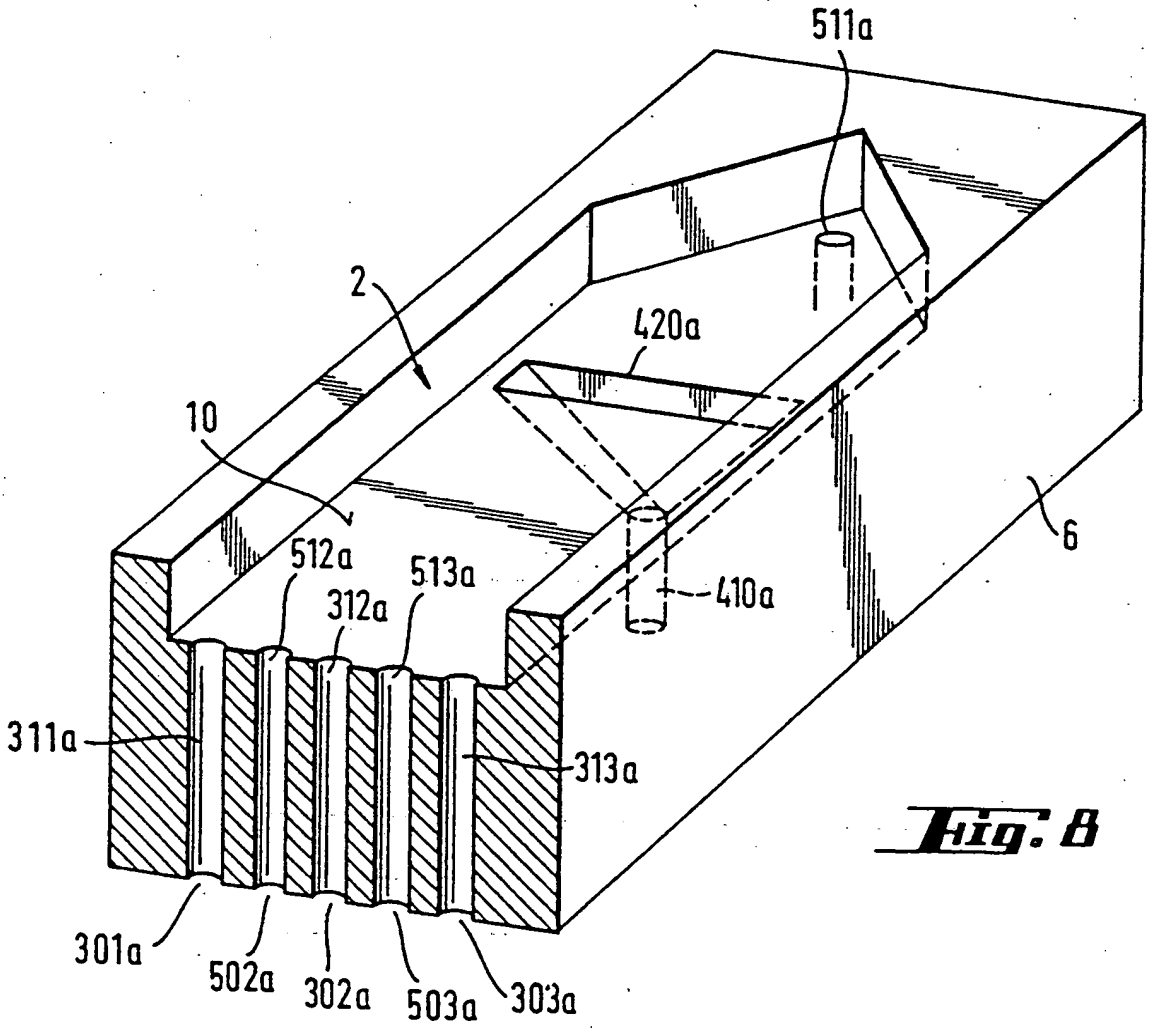

511a
2
420a
10
512a
513a
312a
410a
6
311a
313a
301a
502a
302a
503a
303a

Fig. 8

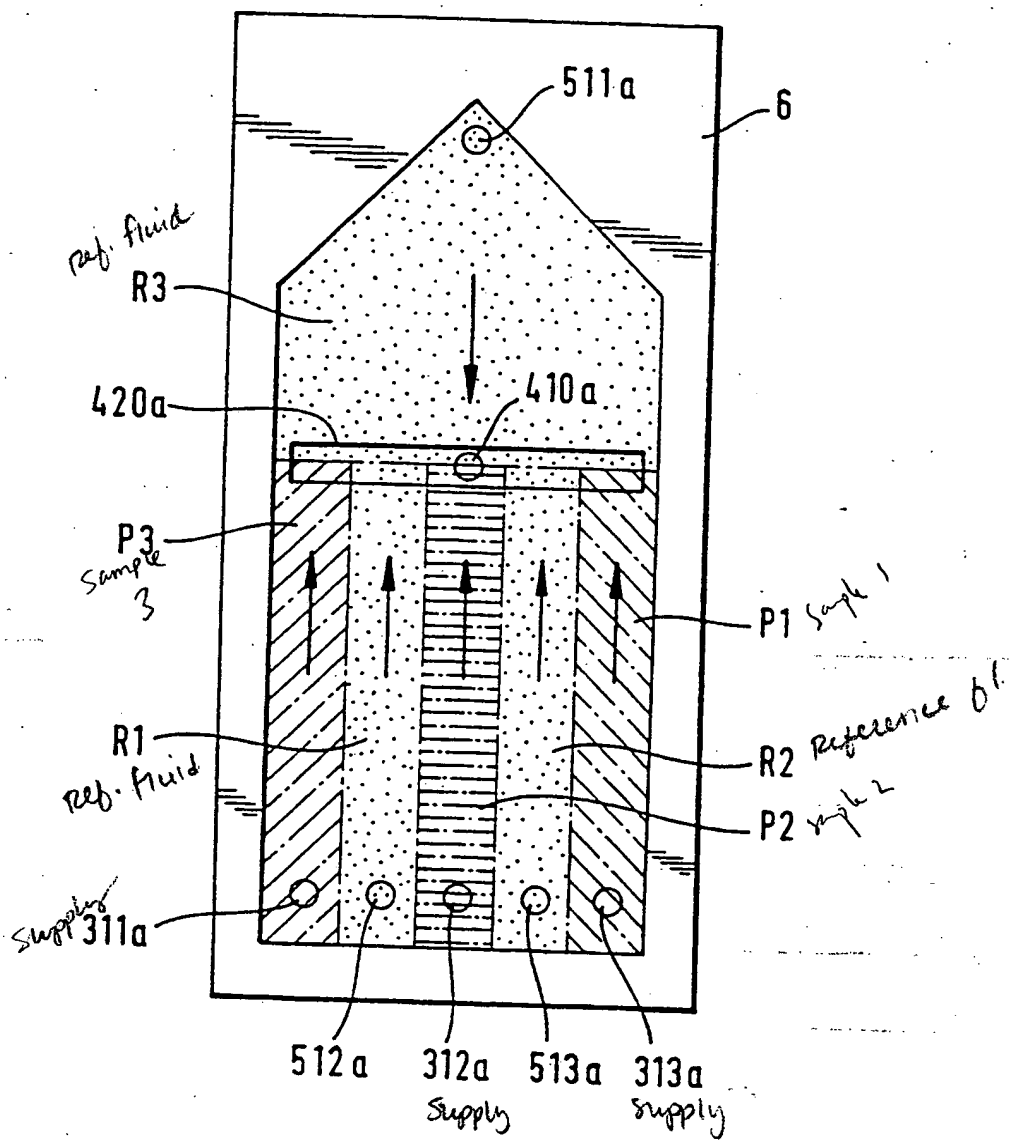

511a
6
ref. fluid
R3
420a
410a
P3
Sample 3
P1 Sample 1
R1
Ref. fluid
R2 Reference fl.
P2 Sample 2
Supply
311a
512a
312a
Supply
513a
313a
Supply

Fig. 9

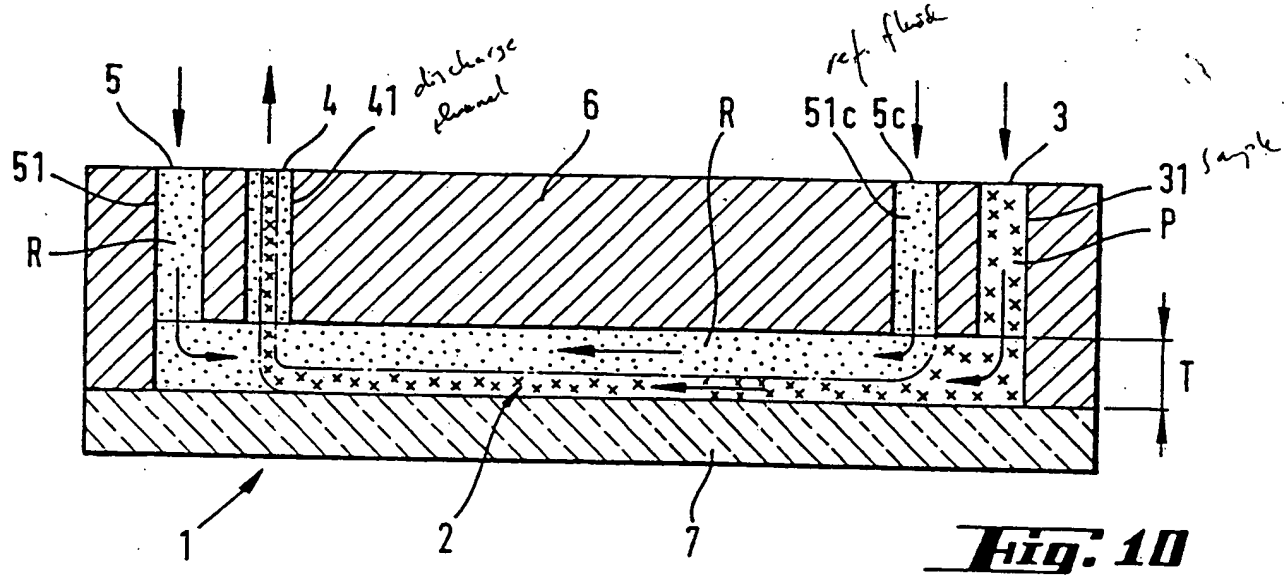

5
4
41
discharge channel
6
R
51c
5c
ref. fluid
3
51
31
sample
R
P
T
1
2
7

Fig. 10

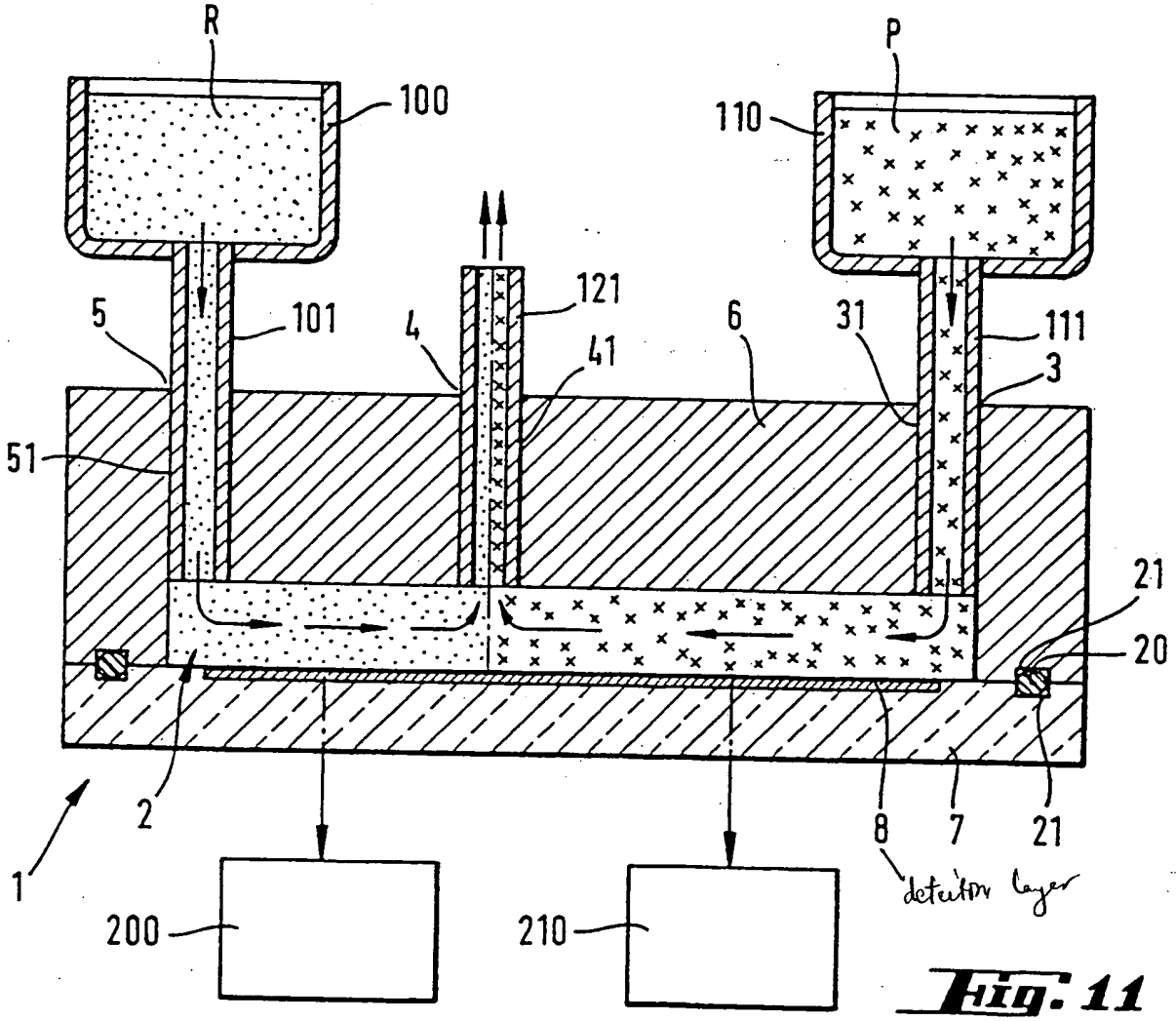
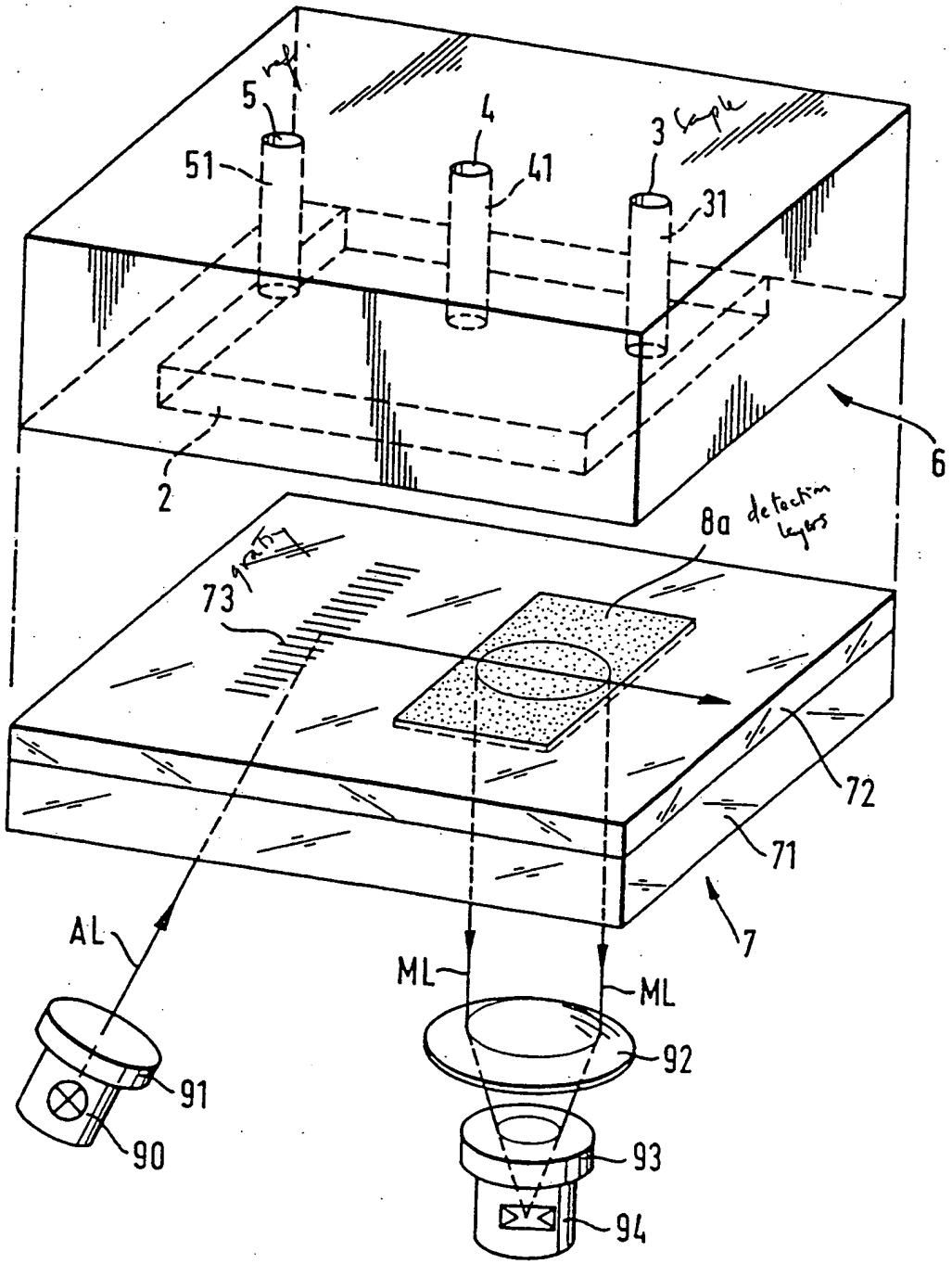

R
100
P
110
121
5
101
4
41
6
31
111
3
51
21
20
2
8
7
21
detection layer
1
200
210

Fig. 11

Fig. 12



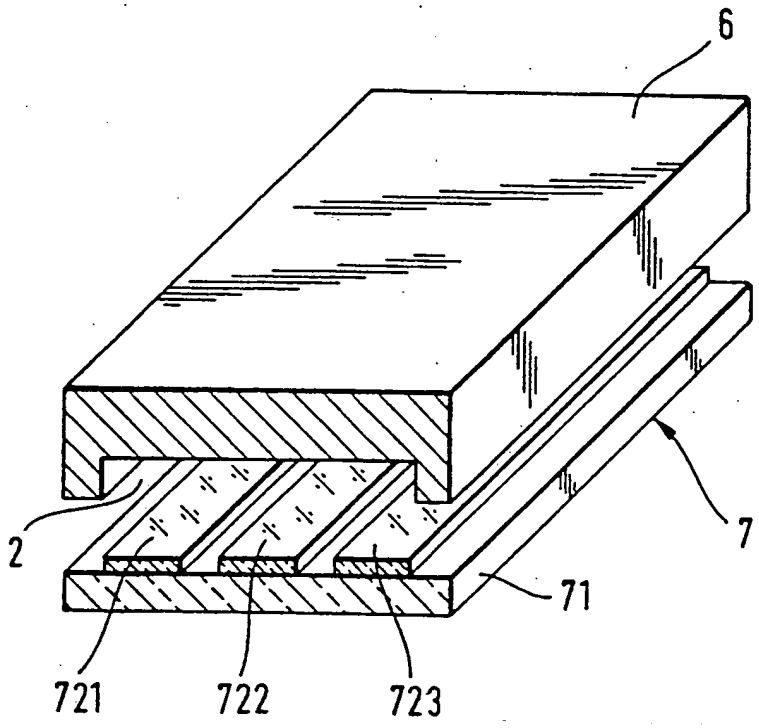


Fig. 13

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/EP 96/02519

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 488 947 (CIBA GEIGY AG) 3 June 1992 see claim 1; figures	1,3,6
A	EP,A,0 257 955 (EMI PLC THORN) 2 March 1988 see column 2, line 24 - column 3, line 22 see column 3, line 40 - column 5, line 27 see claims; figure 4	16,17, 21-23, 26,27,29
A	EP,A,0 482 377 (FRAUNHOFER GES FORSCHUNG) 29 April 1992 see column 2, line 20 - column 4, line 8 see claims; figures	16, 21-23, 26,27,29

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 October 1996	16. 10. 96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk	Authorized officer

1

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter nal Application No
PCT/EP 96/02519

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0488947	03-06-92	DE-D- 59104604	23-03-95
		JP-A- 4268437	24-09-92
EP-A-0257955	02-03-88	JP-A- 63075542	05-04-88
EP-A-0482377	29-04-92	DE-A- 4033912	30-04-92